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DESCRIPTION

FATTY ACID TRANSPORTER PROTEINS AND THE GENES ENCODING THE PROTEINS

Technical Field

The present invention relates to proteins having the activity of transporting fatty acids and the genes encoding the proteins.

Background Art

In the living body, long chain fatty acids such as oleic acid play an important role as an energy source in cellular events including cell membrane synthesis, intracellular signaling, protein modification, transcriptional regulation, etc. Lipid from the diet is first decomposed into fatty acids and glycerols by pancreatic lipase (Chapus C. Biochimie 70: 1223-1234, 1998). Fatty acids are re-esterified and transferred to the lymphoid system (Green P. H. and Aust. N. Z. J. Med. 11: 84-90, 1981). Free fatty acids are also generated through the decomposition of triacylglycerols in fat tissues. Free fatty acids thus generated are present in the circulatory system bound to serum albumin, and are incorporated into fat cells, hepatocytes, cardiac smooth muscle cells, etc. At maximum, 90% of long chain fatty acids are used as an energy source via β-oxidation in intracellular mitochondria, and such.

It is well known that long chain fatty acids are incorporated selectively into the small intestine, hepatocytes, cardiac muscle cells, and fat cells, but the molecular mechanism underlying the transmembrane transport of long chain fatty acids that are incorporated into the cells remains controversial. Several proteins have been suspected as candidates participating in the transport of long chain fatty acids (Ockner R. K. and Kane J. P. J. Biol. Chem. 257: 7872-7878, 1982). Such proteins include the fatty acid transport protein (FATP) that was isolated and cloned by Lodish et al. in 1994.

FATP is a 63-KDa plasma membrane protein isolated from mouse fat cells using the expression cloning strategy. The incorporation of long chain fatty acids was selectively enhanced in animal cells in which the protein was expressed continuously (Shaffer J. E. and

Lodish H. F. Cell 79: 427-436, 1994). Subsequent studies revealed the existence of several homologues of this protein, and thus, mouse FATP-1, -2, -3, -4 and -5 were cloned. Out of them, FATP-1, -2, and -5 are full-length, and were confirmed to be active. However, FATP-3 and -4 are not full-length, and thus, the activity has not been confirmed (Hirsch. D. Proc. Natl. Acad. Sci. 95: 8625-8629, 1998).

FATP is a membrane protein involved in the transport of long chain fatty acids into cells, which is one of the important steps in the metabolic pathway of long chain fatty acids. Previously reported clinical cases of diseases caused by metabolic disorders of long chain fatty acids include myocardial disorders, skeletal muscle disorders, metabolic diseases, renal disorders, neurological dysfunctions, sudden death, etc. (Kelly. D. P. and Strauss A. W. N. Eng. J. Med. 330: 913-919, 1994; Saudubray J. M. In New Developments in Fatty Acid Oxidation, P. M. Coates and K. Tanaka eds., New York: Wiley-Liss). These findings suggest that the malfunction of this membrane protein may be involved in such diseases.

Particularly, in diabetes, insulin deficiency results in the increase of free fatty acids, which contributes to the induction of the diseased state. It is known that the free fatty acid level in blood plasma of diabetics is elevated indeed up to about four times as that of healthy persons after meals, or up to about three times as that of the fasting level of healthy persons. It has also been reported that the expression level of FATP is regulated at the cellular level by insulin and PPARγ (Martin G. J. Biol. Chem. 45: 28210-28217, 1997). Therefore, the above phenomenon could be remedied by enhancing the metabolism of free fatty acid in blood plasma, i.e. enhancing FATP action.

In addition, accumulation of excessive fat causes obesity. The enhancement of FATP action can result in enhanced lipolysis, and thus, is expected to have a therapeutic effect on obesity. On the other hand, this membrane protein is distributed broadly in the human body, including the small intestine, and directly participates in the incorporation of fatty acids from the diet. Thus, for example, obesity can be relieved by suppressing the action of FATP in the small intestine (Andreas Stahl, Molecular Cell, 4, 299-308, 1999). In addition, since

the expression of FATP is induced at the individual level in the LPS-administered sepsis model rat (Memon R. A. Am. J. Physiol. 274 (2 Pt 1), E210-217, 1998), inflammatory diseases including sepsis can be treated by suppressing FATP action.

Further, FATP family members have an ATP-binding region and conserved C-terminal region in the primary structure, and these regions are also conserved in acyl-CoA synthetase. Accordingly, FATP family members are also predicted to have acyl-CoA synthetase activity. Up to now, mouse FATP-1 has been reported to have this activity (Natalie Ribarik Coe, J.B.C., 274, 36300-36304, 1999).

Thus, the FATP family is assumed to participate in not only the transport, but also the metabolism of long chain fatty acids. Accordingly, maintaining the activity of this protein would result in the maintenance of both the transport and metabolism of fatty acids, which is expected to improve diseases caused by disorders in fatty acid metabolism (myocardial disorders, skeletal muscle disorders, metabolic diseases, renal disorders, neurological dysfunction, and sudden death).

Furthermore, in general, human membrane proteins include many proteins that, similar to the tissue plasminogen activator (TPA), are useful as pharmaceuticals by themselves, or like membrane receptors, can be target proteins for pharmaceuticals.

Thus, it is of great significance to provide novel membrane proteins, particularly, membrane proteins associated with the transport and metabolism of fatty acids.

Disclosure of the Invention

An objective of the present invention is to provide membrane proteins having the activity of transporting fatty acids, the genes encoding the proteins, a method for producing them, and uses thereof.

As described above, membrane proteins, particularly those that participate in the incorporation of long chain fatty acids into cells, and compounds capable of regulating the incorporation can be therapeutic agents for diseases associated with disorders in long chain fatty acid metabolism.

Thus, to achieve the above-mentioned objective, the present

inventors conducted strenuous studies aiming at the cloning of novel human genes, as follows. First, clones having a high fullness ratio were isolated from a human cDNA library prepared by the oligo-capping method (Maruyama K. and Sugano S. Gene 138: 171-174, 1994; Suzuki Y. et al. Gene 200: 149-156, 1997). The nucleotide sequences of the obtained cDNA clones having a high fullness ratio were then determined from both the 5' and 3' ends. Then, human full-length cDNAs, which were deduced to be full length cDNA clones, were selected by ATGpr (Salamov A. A. et al. Bioinformatics 14: 384-390, 1998; http://www.hri.co.jp/atgpr/), and such. Based on the nucleotide sequences of human full-length cDNA clones thus obtained, clones that were predicted by PSORT (Nakai K and Kanehisa M. Genomics 14: 897-911, 1992) to have a signal sequence were specifically selected. cDNA clones predicted to encode membrane proteins were thus obtained. The full-length cDNA sequences of the clones were analyzed, and the amino acid sequences were deduced from the nucleotide sequences. Then, GenBank (http://www.ncbi.nlm.nih.gov/Web/Genbank/index.html) and SwissProt (http://www.ebi.ac.uk/ebi_docs/swissprot_db/swisshome.html) were searched for homology to the amino acid sequences deduced by BLAST (Altschul S. F. et al. J. Mol. Biol. 215: 403-410, 1990; Gish W. and States D. J. Nature Genet. 3: 266-272, 1993; http://www.ncbi.nlm.nih.gov/BLAST/).

After such analyses, the present inventors focused on PSEC0067 (hereinafter also referred to as "PSEC67"), which was one of the full-length cDNA clones. This clone was revealed to encode a novel protein having 83% homology to the gene for mouse fatty acid transport protein-3 (FATP-3) at the nucleotide level (Figures 6 to 8), as well as having 83% homology to the protein at the amino acid level (Figure 9). In addition, the present inventors experimentally demonstrated that the protein encoded by PSEC67 indeed had the activity of incorporating long chain fatty acids. The present inventors then found that diseases associated with the long chain fatty acid metabolism could be prevented and treated by regulating the activity of the protein encoded by PSEC67, and thus completed the present invention. Namely, the present invention relates to the following novel membrane proteins,

the genes thereof, a method for producing them), and uses thereof.

[1] A polynucleotide according to any one of the following (a) to (d):

(a) a polynucleotide comprising the protein coding region of the nucleotide sequence of SEQ ID NO: 1,

(b) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2,

(c) a polynucleotide encoding a protein that comprises the amino acid sequence of SEQ ID NO: 2 in which one or more amino acids have been substituted, deleted, inserted, and/or added, and is functionally equivalent to the protein comprising the amino acid sequence of SEQ ID NO: 2, and,

(d) a polynucleotide that (a) hybridizes to a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1 under stringent conditions, and (b) encodes a protein functionally equivalent to the protein comprising the amino acid sequence of SEQ ID NO: 2.

[2] A polynucleotide encoding a partial peptide of a protein encoded by a polynucleotide according to [1].

[3] A protein encoded by a polynucleotide according to [1] or [2].

[4] A vector into which a polynucleotide according to [1] or [2] has been inserted.

[5] A transformant harboring a polynucleotide according to [1] or [2], or the vector according to [4].

[6] A method for producing the protein according to [3], wherein said method comprises the steps of culturing the transformant according to [5] and recovering the expression product.

[7] An antibody against the protein according to [3].

[8] An immunological method for assaying the protein according to [3], wherein said method comprises the step of detecting an immunological reaction between the antibody according to [7] and the protein according to [3].

[9] A polynucleotide comprising at least 15 nucleotides, wherein said polynucleotide comprises a nucleotide sequence complementary to a polynucleotide according to [1], or to a complementary strand thereof.

[10] A primer for synthesizing a polynucleotide according to [1], which comprises the polynucleotide according to [9].

[11] A probe for detecting a polynucleotide according to [1], which comprises the polynucleotide according to [9].

[12] An antisense DNA against a polynucleotide according to [1], or a portion thereof.

[13] A method of screening for a compound binding to the protein according to [3], wherein said method comprises the steps of:

(a) contacting the protein according to [3] with a test sample, and,

(b) selecting a compound binding to the protein.

[14] A compound binding to the protein according to [3], which is isolated by a method as set forth in [13].

[15] A method of screening for a compound regulating the incorporation of a long chain fatty acid to a cell expressing the protein according to [3], wherein said method comprises the steps of:

(a) contacting the cell expressing the protein according to [3] with the labeled long chain fatty acid and a test sample, and incubating the mixture,

(b) measuring the activity of incorporating the long chain fatty acid into the cell, and,

(c) selecting a compound regulating the incorporation activity based on a comparison with the activity measured in the absence of the test sample.

[16] A compound for regulating the incorporation of a long chain fatty acid to a cell expressing the protein according to [3], wherein the compound is isolated by a method as set forth in [15].

The homologies of amino acid sequences and nucleotide sequences of the present invention can be determined using BLAST algorithm by Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90:5873-5877, 1993). Based on this algorithm, programs such as BLASTN and BLASTX have been developed (Altschul et al. J. Mol. Biol. 215:403-410, 1990). When nucleotide sequences are analyzed by BLASTN based on BLAST, the parameters may be, for example, score = 100, and word length = 12. Further, when amino acid sequences are analyzed by BLASTX based on BLAST, the parameters may be, for example, score = 50 ,and word length = 3. When BLAST and Gapped BLAST programs are used, default parameters can be used in the respective programs. Specific techniques used in these analytical methods are well known

(http://www.ncbi.nlm.nih.gov.).

The present invention relates to a novel membrane protein PSEC67. PSEC67 (SEQ ID NO: 2) included in the proteins of the present invention is a membrane protein encoded by a gene obtained by screening cDNAs prepared from the NT-2 neuronal precursor cell, which is a teratocarcinoma cell derived from human fetal testis, of which differentiation had been induced by retinoic acid. This protein is a novel membrane protein having the activity of transporting fatty acids. Thus, the proteins of the present invention, the genes and compounds capable of regulating the activity of the proteins of the present invention are applicable to the prevention and treatment of diseases associated with the metabolism of long chain fatty acids. Further, causes of the diseases can be revealed by detecting abnormalities in the structure or expression level of the genes and proteins of the present invention.

The proteins of the present invention can be prepared as a recombinant or natural proteins. A recombinant protein can be prepared, for example, by introducing into an appropriate host cell a vector into which a DNA encoding the protein of the present invention has been inserted to produce a transformant, and purifying the protein expressed in the transformant, as described below.

On the other hand, a natural protein can be prepared, for example, by using an affinity column in which a below-mentioned antibody against the proteins of the present invention is bound (Current Protocols in Molecular Biology edit. Ausubel et al. (1987) Publish. John Wily and Sons Section 16.1-16.19). The antibody used in the affinity purification may be a polyclonal or monoclonal antibody. Further, the proteins of the present invention can be prepared by *in-vitro* translation (for example, see "On the fidelity of mRNA translation in the nuclease-treated rabbit reticulocyte lysate system. Dasso, M. C., Jackson, R. J. (1989) NAR 17:3129-3144") and such.

The present invention includes a protein comprising a modified amino acid sequence provided by deleting, adding, or inserting one or more amino acids, and/or by substituting one or more amino acids with other amino acids in the amino acid sequence of SEQ ID NO: 2, which is functionally equivalent to the protein comprising the amino

acid sequence of SEQ ID NO: 2. The term "functionally equivalent to the protein comprising the amino acid sequence of SEQ ID NO: 2" means that the protein of interest has biological properties equivalent to PSEC67 protein. The biological properties of PSEC67 protein include the activity of incorporating long chain fatty acids such as oleic acid and arachidonic acid. Namely, the proteins of the present invention are expressed on the surface of cell membranes and have the activity of transporting long chain fatty acids represented by oleic acid or arachidonic acid into cells. The long chain fatty acids transported into cells by the proteins of the present invention include, in addition to the above-mentioned oleic acid and arachidonic acid, fatty acids having 10 to 26 carbon atoms, such as capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and eicosapentaenoic acid. In the present invention, a preferred protein functionally equivalent to PSEC67 has at least 85% or more amino acid identity to the amino acid sequence of SEQ ID NO: 2. In the present invention, the functionally equivalent protein exhibits, specifically 90% or more, more preferably 95% or more amino acid sequence identity. BLAST search algorithm or the like can be used to determine the amino acid sequence identity.

There is no restriction on the number of mutations and mutation sites in the amino acid sequence of a protein, as long as the function of the protein is maintained. The number of mutations (percentage) is typically within 10%, preferably within 5%, and more preferably within 1% of the entire number of amino acids.

Those skilled in the art can prepare proteins functionally equivalent to PSEC67, for example, using a method for introducing mutations into the amino acid sequence of a protein (for example, site-specific mutagenesis (Current Protocols in Molecular Biology edit. Ausubel et al., 1987: Publish. John Wily and Sons Section 8.1-8.5). In addition, spontaneous mutations of amino acids may provide such proteins.

The activity of transporting a fatty acid of a membrane protein into a cell can be verified by a known method. Specifically, the activity can be confirmed by culturing, in the presence of a fatty acid, cells expressing the protein of which the activity is to be

tested, and examining the incorporation of the fatty acid into the cells. The incorporation of a fatty acid can be monitored using a fatty acid labeled with a radioisotope (RI) or the like. Fatty acids used for testing the incorporation include oleic acid and arachidonic acid. Alternatively, instead of these fatty acids, any fatty acid can be used, as long as it has been demonstrated to be incorporated by PSEC67. The incorporation of a fatty acid via PSE6C7 can be verified by culturing cells expressing PSCE67 in the presence of a test fatty acid and examining the incorporation of the test fatty acid into cells. More specifically, an example is the following method:

(1) cells expressing a protein of the present invention are cultured in the presence of charcoal-treated serum,

(2) a complex of BSA and fatty acid labeled with a radioisotope is prepared, and,

(3) the complex is added to the cells, and then, the incorporated fatty acid is detected.

The hybridization technique or the gene amplification technique known to those skilled in the art can be used to isolate a protein functionally equivalent to PSEC67. Specifically, one skilled in the art can isolate DNA exhibiting high homology to the DNA sequence encoding PSEC67 (SEQ ID NO: 1) based on the whole or part of the sequence by using the hybridization technique (Current Protocols in Molecular Biology edit. Ausubel et al., 1987: Publish. John Wily and Sons Section 6.3-6.4), and thus, routinely prepare proteins functionally equivalent to the protein from the isolated DNA. Such proteins encoded by DNA hybridizing to the DNA encoding PSEC67, and which are functionally equivalent to the PSEC67, are also included in the proteins of the present invention.

Organisms from which functionally equivalent proteins are isolated, include, in addition to humans, for example, rats, rabbits, chicken, pigs and cattle, but are not limited thereto.

The hybridization stringency used for isolating a DNA encoding a functionally equivalent protein generally is "1x SSC, 0.1% SDS, 37°C", or the like; more stringent conditions are"0.5x SSC, 0.1% SDS, 42°C", or the like; even more stringent conditions are "0.2x SSC, 0.1% SDS, 65°C", or the like. The more stringent the hybridization

conditions are, the more one can expect to isolate a DNA exhibiting a higher homology to the probe sequence. However, the above-mentioned combinations of conditions such as SSC, SDS, and temperature, are merely examples. Those skilled in the art can achieve a similar stringency by appropriately combining the above-mentioned factors, or other factors (for example, probe concentration, probe length, duration of hybridization, etc.) that determine the hybridization stringency.

Proteins isolated by using such a hybridization technique generally have amino acid sequences having a high homology to PSEC67. The term "high homology" means at least 85% or more, preferably 90% or more, or more preferably 95% or more sequence identity.

Alternatively, a DNA fragment, which exhibits a high homology to the whole or part of the DNA sequence encoding PSEC67, is isolated by a gene amplification technique (PCR; Current protocols in Molecular Biology edit. Ausubel et al., 1987: Publish. John Wiley and Sons (Section 6.1-6.4) using primers designed based on any part of the DNA sequence encoding PSEC67 (SEQ ID NO: 1). Then, a protein functionally equivalent to PSEC67 protein can be obtained by using the DNA fragment.

The present invention also includes partial peptides of the proteins of the invention. A partial peptide comprises a protein in which a signal peptide has been removed. In addition, the present invention comprises an antigen peptide for raising antibodies. For the peptides to be specific for the proteins of the invention, the peptides should comprise at least 7 amino acids, preferably 8 amino acids or more, and more preferably 9 amino acids or more. The peptides can be used for preparing antibodies against the proteins of the invention, or competitive inhibitors of them, and also screening for receptors that binds to the proteins of the invention. Partial peptides of the invention can be produced, for example, by genetic engineering methods, known peptide-synthesis methods, or by digesting the proteins of the present invention with an appropriate peptidase.

The present invention also relates to polynucleotides encoding the proteins of the invention. The polynucleotides of the invention can be provided in any form, as long as they encode the proteins of the invention, and thus includes, cDNA, genomic DNA, chemically

synthesized DNA, RNA, etc. The polynucleotides also include a those comprising any nucleotide sequence obtained based on the degeneracy of the genetic code, as long as they encode the proteins of the invention. The polynucleotides of the invention may be fused with polynucleotides encoding other proteins or oligopeptides. A polynucleotide of the invention can be isolated by standard methods such as hybridization using a probe DNA comprising the whole or part of the nucleotide sequence encoding PSEC67 (SEQ ID NO: 1), or by PCR using primers that are synthesized based on these nucleotide sequence.

The present invention also relates to a vector into which a polynucleotide of the invention is inserted. The vector of the invention is not limited as long as it contains the inserted polynucleotide stably. For example, if *E. coli* is used as a host, a vector such as the pBluescript vector (Stratagene) is preferable as a cloning vector. To produce a protein of the invention, expression vectors are especially useful. Any expression vector can be used as long as it is capable of expressing the protein *in vitro*, in *E. coli*, in cultured cells, or *in vivo*. For example, pBEST vector (Promega) is preferable for *in vitro* expression, pET vector (Invitrogen) for *E. coli*, pME18S-FL3 vector (GenBank Accession No. AB009864) for cultured cells, and pME18S vector (Mol. Cell. Biol. (1988) 8: 466-472) for *in vivo* expression. To insert a polynucleotide of the invention, ligation utilizing restriction sites can be performed according to the standard method (Current Protocols in Molecular Biology (1987) Ausubel et al. edit, John Wily & Sons, Section 11.4-11.11).

The present invention also relates to a transformant harboring a vector of the invention. Any cell can be used as a host into which a vector of the invention is introduced, and various kinds of host cells can be used depending on the purpose. For strong expression of a protein in eukaryotic cells, for example, COS cells or CHO cells can be used,

Calcium phosphate precipitation method, electroporation method (Current Protocols in Molecular Biology (1987) Ausubel et al. edit, John Wily & Sons, Section 9.1-9.9), lipofectamine method (GIBCO-BRL), microinjection method, etc can be used to introduce the vector into the host cells.

Furthermore, the present invention provides a polynucleotide comprising at least 15 nucleotides and complementary to the polynucleotide of SEQ ID NO: 1 or its complementary strand. As used herein, the term "complementary strand" refers to one strand of a double-stranded polynucleotide which forms base pairs of A:T (A:U) and G:C with the other strand of the polynucleotide. Also, "complementary" is defined as not only sequences that completely match a continuous nucleotide region of at least 15 nucleotides, but also sequences having a homology of at least 70%, preferably 80% or more, more preferably 90% or more, and most preferably 95% or more to that region. The homology may be determined using the algorithm described herein. Such a polynucleotide can be used as a probe for isolating and detecting a polynucleotide of the invention, and as a primer for amplifying a polynucleotide of the present invention. When used as a primer, the polynucleotide usually comprises 15 to 100 bp, and preferably of 15 to 35 bp. When used as a probe, the polynucleotide comprises the whole or a part of the sequence of a polynucleotide of the invention, and comprises at least 15 bp.

The polynucleotides of the present invention can be used for examining and diagnosing abnormalities of the proteins of the invention. For example, it is possible to examine an abnormal expression of a gene encoding a protein using a polynucleotide of the invention as a probe for northern hybridization, or as a primer for RT-PCR. Also, a polynucleotide of the invention can be used as a primer for a polymerase chain reaction (PCR), such as genomic DNA-PCR and RT-PCR, to amplify a DNA encoding a protein of the invention or the expression regulatory region, and then examine and diagnose sequence abnormalities using RFLP analysis, SSCP, and sequencing, etc.

Furthermore, a "polynucleotide comprising at least 15 nucleotides that specifically hybridizes to the polynucleotide of SEQ ID NO: 1", includes an antisense DNA for suppressing the expression of a protein of the invention. To exert an antisense effect, an antisense DNA has at least 15 bp or more, preferably 100 bp, and more preferably 500 bp or more, and usually has 3000 bp or less, and preferably 2000 bp or less. Antisense DNAs can be used in the gene therapy of diseases (especially, diseases involved in the metabolism of long

chain fatty acids) caused by abnormalities of the proteins of the invention (abnormal function or abnormal expression). An antisense DNA can be prepared, for example, by the phosphorothioate method ("Physicochemical properties of phosphorothioate oligodeoxynucleotides." Stein (1988) Nucleic Acids Res. 16: 3209-3221) based on the sequence information of a polynucleotide encoding a protein of the invention (for example, the DNA of SEQ ID NO: 1).

The polynucleotides or antisense DNAs of the present invention can be used in gene therapy, for example, by administrating them to patients by the *in vivo* or *ex vivo* method using virus vectors such as retrovirus vectors, adenovirus vectors, and adeno-associated virus vectors, or non-virus vectors such as liposomes.

The present invention also relates to antibodies that bind to the proteins of the invention. There are no limitations to the form of the antibodies of the invention, and include, polyclonal antibodies, monoclonal antibodies, or portions thereof that can bind to the antigens. They also include antibodies of all classes. Furthermore, specialized antibodies such as humanized antibodies are also included.

A polyclonal antibody of the invention can be obtained according to the standard methods for synthesizing an oligopeptide corresponding to the amino acid sequence and immunizing rabbits with the peptide (Current Protocols in Molecular Biology (1987) Ausubel et al. edit, John Wiley & Sons, Section 11.12-11.13). A monoclonal antibody of the invention can be purified according to standard methods for purifying proteins expressed in *E. coli*, immunizing mice with the protein, and producing a hybridoma cell by fusing the spleen cells and myeloma cells (Current Protocols in Molecular Biology (1987) Ausubel et al. edit, John Wiley & Sons, Section 11.4-11.11) .

An antibody binding to a protein of the present invention can be used for purifying the protein, and also for examining and diagnosing abnormalities in the expression and structure of the protein. Specifically, proteins can be extracted, for example, from tissues, blood, or cells. The proteins of the invention are detected by Western blotting, immunoprecipitation, ELISA, and so on, for the above purpose.

Furthermore, the antibodies binding to the proteins of the present invention can be utilized for treating diseases associated

with the proteins of the invention. If the antibodies are used for treating patients, human antibodies or humanized antibodies are preferable in terms of their low antigenicity. Human antibodies can be prepared by immunizing a mouse whose immune system is replaced with that of humans (for example, "Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice" Mendez M.J. et al. (1997) Nat. Genet. 15: 146-156). Humanized antibodies can be prepared by recombination of the hypervariable region of a monoclonal antibody (Methods in Enzymology (1991) 203: 99-121).

Further, the present invention relates to a method for screening a compound binding to a protein of the present invention using a protein of the present invention. The screening method comprises the steps of: (a) contacting a test sample with a protein of the present invention or a partial peptide thereof, and (b) selecting a compound binding to the protein or partial peptide.

Compounds selected by the screening method is expected to regulate the activity of incorporating fatty acids into cells by the proteins of the present invention.

Specific examples of such methods include a method for purifying a compound by contacting a test sample with an affinity column where a protein of the present invention has been immobilized, a method using the two-hybrid system, West-Western blotting, and a high-throughput screening method in the combinatorial chemistry technique.

The test samples used for the screening include, for example, cell extracts, expression products of a gene library, synthetic low molecular weight compounds, synthetic peptides and natural compounds, but are not limited thereto.

Radioisotopes such as ^{125}I, and enzymes (alkaline phosphatase, etc.) can be used as labels. Further, the detection can be achieved by labeling an antibody against a protein of the present invention instead of labeling the protein.

In addition, compounds regulating the incorporation activity can be screened using as an index the activity of incorporating long chain fatty acids into cells by the membrane proteins of the present invention.

This screening method comprises the steps of: (a) contacting the cell expressing the protein of the present invention with the labeled long chain fatty acid and a test sample, and incubating the mixture, (b) measuring the activity of incorporating the long chain fatty acid into the cell, and (c) selecting a compound regulating the incorporation activity based on a comparison with the activity measured in the absence of the test sample.

The test samples used for the screening include, for example, cell extracts, expression products of a gene library, synthetic low molecular weight compounds, synthetic peptides and natural compounds, but are not limited thereto. In addition, it is possible to use as a test sample a compound isolated by the screening using as an index the binding activity to a protein of the present invention.

The long chain fatty acid used for the screening includes oleic acid, arachidonic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and eicosapentaenoic acid. Particularly, oleic acid or arachidonic acid is used preferably. Further, any fatty acid that has been verified to be incorporated by PSEC67 can be used for the screening method of the present invention.

The labeling of the above-mentioned long chain fatty acid includes a labeling with the above-mentioned RI. When RI labeling is used, the above-mentioned incorporation activity can be assayed by measuring the radioactivity contained in the cells using a scintillation counter, or the like. When the determined value is significantly greater than that in the absence of the test sample, it is assessed that the incorporation activity has been enhanced. On the other hand, when the determined value is significantly lower than that in the absence of the test sample, it is assessed that the incorporation activity has been inhibited.

Compounds screened by the above-mentioned method, particularly compounds enhancing the incorporation of fatty acids, are expected to have therapeutic effects on diabetes and arteriosclerosis. On the other hand, compounds inhibiting the incorporation of fatty acids can be expected to have therapeutic effects as anti-obesity drugs. Further, the compounds are applicable as preventive or therapeutic

agents for diseases associated with the proteins of the present invention (for example, metabolic diseases such as diabetes, arteriosclerosis, and obesity, cardiomyopathy, skeletal muscle disorders, renal disorders, etc.).

When a compound isolated by the screening method of the present invention is used as a pharmaceutical, the isolated compound can be administered by itself directly to a patient or alternatively administered after being formulated by a known pharmaceutical method. For example, the compound can be administered after being formulated by appropriately combining with a pharmaceutically acceptable carrier or solvent, specifically, sterilized water, physiological saline, vegetable oil, an emulsifier, suspension, etc. A method known to those skilled in the art, for example, intra-arterial injection, intravenous injection, subcutaneous injection, etc., can be used to administer the compound or formulation to a patient. While the dose depends on the weight and age of the patient, the administration method, and such, those skilled in the art can suitably select an appropriate dose. Further, when the compound is encoded by a DNA, gene therapy can be achieved by inserting the DNA into a gene therapy vector. While the dose and administration method depends on the weight and age of the patient, symptoms, and so on, those skilled in the art can suitable select a dose. All the cited literatures of prior art are incorporated herein by reference in their entirety.

Brief Description of the Drawings

Figure 1 shows the expression of PSEC67 protein in 293 cells.

Figure 2 shows the incorporation of oleic acid into transiently expressing cells . The vertical axis indicates the rate of oleic acid incorporation when compared with that into the mock-transfected cells.

Figure 3 shows the incorporation of oleic acid into constitutively expressing cells. The numerals in the horizontal axis indicate clone numbers. The vertical axis indicates the degree of fatty acid incorporation (fold) relative to that of the mock-transfected clone.

Figure 4 shows tissue distribution of PSEC67 determined by

RT-PCR.

Figure 5 shows the incorporation of oleic acid or arachidonic acid. The vertical axis indicates the ratio determined by taking the value of the buffer in the mock-transfected cells as one. Oleic acid was used in (A) and arachidonic acid was used in (B).

Figure 6 shows a comparison between the nucleotide sequences of PSEC67 of the present invention and the cDNA encoding mouse FATP-3.

Figure 7 shows a comparison between the nucleotide sequences of PSEC67 of the present invention and the cDNA encoding mouse FATP-3.

Figure 8 shows a comparison between the nucleotide sequences of PSEC67 of the present invention and the cDNA encoding mouse FATP-3.

Figure 9 shows a comparison between the amino acid sequences of PSEC67 of the present invention and mouse FATP-3.

Figure 10 shows the alignment of the amino acid sequences of PSEC67 of the present invention (top) and human acyl-CoA synthetase (bottom). The sequences underlined, YIFTSGTTGLPK and FHDRTGDTFRWKGENVATTEVA, indicate ATP-binding region and conserved FATP region, respectively.

Best Mode for Carrying out the Invention

The present invention is illustrated in detail below with reference to Examples, but it is not to be construed as being limited thereto.

[Example 1] Isolation of PSEC67

A cDNA library was prepared by using NT-2 neuronal precursor cells (purchased from Stratagene), which is a teratocarcinoma cell line derived from human fetal testis that can be differentiated to nerve cells by treating with retinoic acid. First, NT-2 cells were cultured in the presence of retinoic acid according to the attached manual, the cultured cells were harvested, and mRNA was extracted from them according to a method described in references (J. Sambrook, E. F. Fritsch & T. Maniatis, Molecular Cloning Second edition, Cold Spring harbor Laboratory Press 1989). Further, poly(A)$^+$ RNA was purified with oligo dT cellulose.

A cDNA library was prepared from poly(A)$^+$ RNA by the oligo-capping

method (M. Maruyama and S. Sugano, Gene, 138: 171-174 (1994)). Using an oligo-cap linker (agcaucgagu cggccuuguu ggccuacugg/SEQ ID NO: 3) and oligo dT primer (gcggctgaag acggcctatg tggccttttt ttttttttttt tt/SEQ ID NO: 4), BAP (bacterial alkaline phosphatase) treatment, TAP (tobacco acid phosphatase) treatment, RNA ligation, first strand cDNA synthesis, and RNA removal were performed according to methods described in references (Suzuki and Sugano, Protein, Nucleic Acid and Enzyme, 41: 197-201 (1996); Y. Suzuki et al., Gene, 200: 149-156 (1997)). Then, the resultant single-stranded cDNA was converted to double-stranded cDNA by PCR (polymerase chain reaction) using a 5' PCR primer (agcatcgagt cggccttgtt g/SEQ ID NO: 5) and a 3' PCR primer (gcggctgaag acggcctatg t/SEQ ID NO: 6), and then digested with SfiI. Next, a cDNA library was constructed by inserting the cDNA uni-directionally into DraIII-digested vector pME18SFL3. Complementary DNA clones whose cDNA insert sizes were 1 kb or shorter were removed from the pool of clones obtained from the library Then, the nucleotide sequences of the 5' and 3' ends of cDNA inserts in the plasmid DNAs were determined using a DNA sequencer (ABI PRISM 377; PE Biosystems) following a sequencing reaction using a DNA sequencing reagent (Dye Terminator Cycle Sequencing FS Ready Reaction Kit, dRhodamine Terminator Cycle Sequencing FS Ready Reaction Kit or BigDye Terminator Cycle Sequencing FS Ready Reaction Kit; PE Biosystems), according to the manual.

The fullness ratio of the 5' end of the cDNA from the library prepared by the oligo-capping method was estimated by the following method: All the clones whose 5' end sequences were shared by known human mRNAs in public databases, were assessed to be "full-length" when the 5' end sequences were longer than the known mRNA sequences , or even when the 5' ends were short, but had a translation initiation codon. The clones were alternatively assessed to be "not full-length" when they had no translation initiation codons. The fullness ratio at the 5' end [the number of full-length clones / (the number of full-length clones + the number of not full-length clones)] of each cDNA clone from the library was determined based on a comparison with human known mRNAs. The result showed that the fullness ratio of the library was 62%, and thus, the fullness ratio at the 5' end sequence

was revealed to be very high.

Then, the fullness ratio at the 5′ end of cDNA was assessed by ATGpr and ESTiMateFL.

ATGpr is a program that predicts a translation initiation codon based on characteristics of sequences in the vicinity of ATG codon, which was developed by A. A. Salamov, T. Nishikawa, and M. B. Swindells at the Helix Research Institute (A. A. Salamov, T. Nishikawa, M. B. Swindells, Bioinformatics, 14: 384-390 (1998); http://www.hri.co.jp/atgpr/). The result was shown as an expectation value (0.05-0.94) for when ATG is the true start codon. The ATGpr1 value of PSEC67 was 0.26.

ESTiMateFL is a method for selecting a clone that is very likely a full-length cDNA by comparing with the 5′-end or 3′-end sequences of ESTs in public databases, which was developed by Nishikawa and Ota of the Helix Research Institute.

By the method, a cDNA clone is judged to be "not very likely to be full length" if there is any EST that is longer than the 5′-end or 3′-end sequence of the clone. This method was systematized for high throughput analysis. A clone is judged to be full length if the clone has a longer 5′-end sequence than ESTs in public databases. Even if a clone has a shorter 5′-end, the clone is judged to be full length if the difference in length is within 50 bases, and otherwise judged to be not full length, for convenience. The estimation precision through EST comparison increases with the increase in the number of ESTs compared. However, when a limited number of ESTs are available, the reliability becomes low. Thus, the method is applicable to efficiently remove clones among the cDNA clones synthesized by the oligo-capping method (fullness ratio at the 5′-end sequence is about 60%) that are not likely to be full length. In particular, the ESTiMateFL is especially useful to estimate the completeness of the 3′-end sequence of cDNA of a human unknown mRNA having a significant number of EST deposits in public databases.

As a result, PSEC67 were predicted to be a full-length cDNA.

From the clones in each library constructed by the oligo-capping method, those clones predicted to have a signal sequence (most likely to be secretory or membrane proteins) were specifically selected by

analyzing the amino acid sequence predicted by all the ATG codons within the 5'-end sequence, for the presence of a signal peptide, which is a characteristic of the N-terminus of many secretory proteins, by using PSORT developed by Nakai and Kanehisa that predicts the localization of a protein (K. Nakai & M. Kanehisa, Genomics, 14: 897-911 (1992)).

The complete cDNA sequence and the predicted amino acid sequence of the clones thus selected were determined. The nucleotide sequence was finally determined by using a combination of the following three methods, assembling the determined sequences, and obtaining a completely overlapped sequence.

(1) Long read sequencing from both ends of the cDNA inserts using a Licor DNA sequencer (Sequence reactions were performed according to the manual of the Licor sequencer (Aloka), and DNA sequence was determined by the sequencer.)

(2) Nested sequencing by the Primer Island method that utilizes the *in vitro* integration reaction of AT2 transposon (Devine S.E., and Boeke J.D. (1994) Nucleic Acids Res. 22: 3765-3772) (Clones were obtained using a kit from PE Biosystems, and sequence reactions were performed using the DNA sequencing reagents from the same company, according to the manufacturer's instructions, and the DNA sequence was determined using an ABI PRISM 377 sequencer).

(3) Primer walking by the dideoxy terminator method using custom synthesized DNA primers (Sequence reactions were performed using the DNA sequencing reagents from PE Biosystems and custom synthesized DNA primers, according to the manufacturer's instructions. DNA sequence was determined using an ABI PRISM 377 sequencer).

These sequences were subjected to analysis by ATGpr and PSORT and also to the BLAST search of GenBank and SwissProt. As a result, most clones were predicted to be secretory protein or membrane proteins that have a signal sequence in the N-terminus. Furthermore, those clones, including PSEC67, in which a signal sequence was not found by PSORT were subjected to the analyses by MEMSAT (D. T. Jones, W. R. Taylor & J. M. Thornton, Biochemistry, 33: 3038-3049 (1994)) and SOSUI (T. Hirokawa et al., Bioinformatics, 14: 378-379(1998)) (Mitsui Knowledge Industry) for the identity as membrane proteins (having

a transmembrane helix). The result predicted that PSEC67 may have a transmembrane helix, and thus was presumed to be a membrane protein. Accordingly, PSEC67, though it has no signal sequence at the N-terminus, was thought to be a membrane protein encoded by a full-length cDNA clone. Further, BLAST analysis using GenBank and SwissProt showed that PSEC67 had 83% homology to mouse fatty acid transport protein-3 (FATP-3) at the nucleotide level and encoded a novel protein having 83% homology to FATP-3 at the amino acid level.

Further, in the primary structure, PSEC67 comprises an ATP-binding region and C-terminal region conserved in FATP family members. The regions are also conserved in the primary structure of acyl-CoA synthetase (Figure 10). These findings suggest that PSEC67 may also have an acyl-CoA synthetase activity. This activity can be verified according to the method of Jae-Yeon Choi et al. (Jae-Yeon Choi, J.B.C., 274, 4671-4688, 1999). The results are shown below. The following data are discriminated with "//" after each clone name.
Clone name (PSEC number)//
Sequence name//
Size of cDNA (bp)//
The number of amino acids (a.a.) in the deduced amino acid sequence //
The number of ATG codons counted from the N terminus//
Maximal ATGpr1 value//
The presence or absence of a signal sequence and result of the prediction for membrane protein by MEMSAT and SOSUI//
Annotation
PSEC67 // C-NT2RP2001142 // 2405 // 730 / / 1 // 0.26 // No & transmembrane // 1462/1751 (83%) similarity to mouse fatty acid transport protein-3
PSEC67 // C-NT2RP2001142 // 2405 // 730 / / 1 // 0.26 // No & transmembrane // 935/1603 (58%) similarity to human very-long-chain acyl-CoA synthase (VLCS)

[Example 2] Construction of expression plasmid "pcDNA 3.1(-) - PSEC67"

The coding region of PSEC67 was ligated to pcDNA3.1(-). First, primers were designed as follows.
Primer 1; 5'-GGAATTCCGTGGAGTGGTGGGGGCCTGGGTGGGAAT-3' (SEQ ID NO: 7)

Primer 2; 5'-CGGGATCCCACCTGCAACGGCCCCCACCCCACAGAGTTC-3' (SEQ ID NO: 8)

The fragment was amplified by PCR using pME18SFL3-PSEC67 as a template and LA Taq polymerase (Takara). After both this cDNA fragment and vector pcDNA3.1 (-) (INVITROGEN) were double-digested with BamHI and EcoRI, the respective DNAs were purified by agarose gel electrophoresis, and then ligated together by using a Takara Ligation kit ver.2. An expression plasmid was prepared by introducing the ligated product into *E. coli* DH5 alpha. The nucleotide sequence of this plasmid was examined.

[Example 3] Construction of plasmid "pcDNA 3.1(-)/MycHis - PSEC67" for testing the expression

The coding region of PSEC67 was ligated to (to) pcDNA 3.1(-)/MycHis-A. First, primers were designed as follows.
Primer 1; 5'-GGAATTCCGTGGAGTGGTGGGGGCCTGGGTGGGAAT-3' (SEQ ID NO: 9)
Primer 3; 5'-CGGGATCCGATTCGAAGGTTTCCTGCCAG-3' (SEQ ID NO: 10)

Then, the fragment was amplified by PCR using pME18SFL3-PSEC67 as a template and LA Taq polymerase (Takara). After both this cDNA fragment and vector pcDNA3.1 (-)/MycHis-A (INVITROGEN) were double-digested with BamHI and EcoRI, the respective DNAs were purified by agarose gel electrophoresis, and then ligated together using a Takara Ligation kit ver.2. A MycHis tagged plasmid for testing the expression was prepared by introducing the ligated product into *E. coli* DH5 alpha. The nucleotide sequence of this plasmid was examined.

[Example 4] Verification of expression

(1) Introduction of plasmid

The expression plasmid was introduced into animal cells such as COS-7 cells and 293 cells by methods such as the calcium-phosphate method, lipofectamine method, or the like.

Plasmid transfection to COS-7 cells using the calcium-phosphate method was carried out as follows:

First, on the first day, the cells were plated on a 10-cm dish at a cell density of 1,000,000 cells/dish, and then cultured in 20 ml of a culture medium at 37°C for 20 hours. 10 μg of the expression

plasmid was dissolved in 983 µl of 0.1x TE, further 142 µl of $CaCl_2$ solution was added thereto. The mixture was cooled on ice. 1.125 ml of ice-cold 2x HBS was prepared in a separate tube, and then 1.125 ml of the DNA mixture was added thereto. After a 2.25-ml aliquot of the solution was added to the dish and mixed well, the cells were incubated at 37°C for 6 hours and subjected to the glycerol-shock treatment. Specifically, after washing twice with 5 ml of TBS, the cells were washed with 5 ml of TBS-20% glycerol. After the cells were further washed twice with 5 ml of TBS and once with 5 ml of PBS(-), 10 ml of culture medium was added thereto and the cells were cultured at 37°C for 20 hours. The culture medium was changed with a fresh one, and then the cells were further cultured at 37°C under an atmosphere of 5% CO_2. In the case of 293 cells, the glycerol-shock treatment was omitted and the culture medium was changed on the following day. On the third day after the introduction, the culture supernatant and cells were obtained. The composition of the solution used is as follows:

2x HBS:

HEPES 5.96 g

NaCl 8.18 g

0.1 M Na_2HPO_4 7.5 ml/500 ml water (pH 7.12)

TBS (per 1 L):

NaCl 8 g

KCl 0.38 g

$Na_2HPO_4/12H_2O$ 0.2 g

Tris-Cl 3 g

$CaCl_2/2H_2O$ 0.114 g

$MgCl_2/6H_2O$ 0.115 g

(2) Sample preparation

After washing twice with PBS (-) buffer, the cells were collected by centrifugation (500x g). 200 µl of a homogenizing buffer (20 mM Tris-Cl, 1 mM A-PMSF) was added to the cell pellet, and the freeze-thaw treatment was repeated three times. Then, the cells were disrupted with 10 strokes of a Teflon homogenizer on ice. The cell lysate was centrifuged at 1000x g, and the cell debris was removed. The supernatant fraction was saved as a cellular fraction. The culture

medium after culture without any treatment, or a sample concentrated with a membrane filter with a cut-off size of molecular weight of 10,000 (CENTRICON; AMICON), was used as the culture supernatant fraction. The expression was examined by Western blotting using 8 μl of samples of the cellular fraction and culture supernatant fraction. 8 μl of the cellular fraction corresponded to approximately 15 μg of protein. Western blotting was carried out as follows.

(3) Protein detection (Western blotting)

2 μl of 5x loading buffer was added to 8 μl of a sample, and the protein was denatured at 100°C for 5 minutes. Then, the solution was cooled on ice for 2 minutes. This sample was subjected to SDS-PAGE (PAGEL, ATTO Corporation) with a current of 20 mA for about one hour. After electrophoresis, the gel was equilibrated in a blotting buffer for about 20 minutes. The proteins in the gel was electro-transferred onto a PVDF membrane pre-equilibrated in same buffer for about 10 minutes with a TRANS-BLOT SD SEMI-DRY TRANSFER CELL (BIO RAD).

Blotting buffer (per 1 L):

3 g Tris-base

14.4 g Glycine

20% Ethanol

The PVDF membrane with transferred protein was subjected to blocking with Block Ace (Dainippon Pharmaceutical) at room temperature for 2 hours, or at 4°C overnight, and then a primary antibody (anti-myc antibody, INVITROGEN) was reacted therewith for 1 hour. After the membrane was washed, a secondary antibody (Peroxidase-conjugated AffiniPure Goat Anti-Mouse IgG; Jackson Immuno Research) was reacted therewith at room temperature for 1 hour. After the membrane was washed, the protein was detected by ECL Western blotting detection reagents (Pharmacia).

As a result, a band of about a 70-Kda protein was found for the cellular fraction from cells in which PSEC67 had been introduced. No corresponding band was observed for the culture supernatant fraction, and for the cellular fraction and culture supernatant fraction of samples from mock-transfected cells (293 cells in which the vector alone had been introduced). Thus, the expression of PSEC67 protein in the cellular fraction was verified (Figure 1).

[Example 5] Assay for the activity of incorporating oleic acid

In order to test the activity of incorporating fatty acids, the incorporation of [^{14}C]-labeled oleic acid into recombinant cells was examined. As described above, the plasmid was introduced into 293 cells in a 15-cm dish using the calcium-phosphate method or lipofectamine method, and the culture medium was changed with a fresh one on the following day. The culture medium was changed with D-MEM (antibiotics -) containing 10% CS without fatty acid the day before the assay, and the cells were cultured for one day. First, albumin-bound fatty acid was prepared on the day scheduled for the assay. 10 µl of [^{14}C]-oleic acid was added to 100 µl of Milli-Q water incubated at 40°C, and the mixture was gently stirred. A BSA solution without fatty acids (prepared from fr. V, SIGMA, 20 g/100 ml) was added to the mixture at a final molar ratio of 1:1, and the mixture was stirred gently. Twice as much volume of PBS(-) was added to the mixture, and the resultant solution was incubated at 37°C for 45 minutes. The culture supernatant was sucked off and the cells were washed twice with 5 ml of PBS(-). 5 ml of PBS(-) was added to the dish, and then the cells were scraped off with a cell scraper and transferred to a tube. After the cells were collected by centrifugation, the cell density was adjusted to 5×10^5 cells/ml by adding PBS(-) thereto. Then, 200-µl aliquots of the cell suspension were transferred into 15-ml polypropylene tubes on ice. After a pre-incubation of the cell suspension at 37°C for five minutes, 50 µl of albumin-bound fatty acid (albumin-bound [^{14}C]-oleic acid solution) was added to each tube, and the tubes were incubated at 37°C for 3 minutes. After the reaction, 5 ml of a stop solution (= Washing solution; 0.1% BSA without fatty acid, 200 µM phloretin in PBS (-)) that was pre-cooled on ice was added to each tube. After the reaction was stopped, the solution was filtered through a GF/C glass filter saturated with a saturate buffer (0.1% BSA (without fatty acid) in PBS(-)) overnight, and then the cells were washed 3 times with 5 ml of a washing solution. Then, this glass filter was soaked in a liquid scintillation vial containing 10 ml of Clear-sol 1 overnight, and the radioactivity was measured.

The result showed that oleic acid incorporation by cells to which

PSEC67 had been transiently introduced was significantly enhanced as compared with that in the mock-transfected cells (Figure 2). The amount of oleic acid incorporated into the control cells to which rat FATP-1 had been introduced, was also increased. When a vector with no insert was introduced into 293 cells (mock transfection), the amount of oleic acid incorporated into the cells was also increased. This increase was presumed to be due to the fatty acid transport protein originally expressed at a low level in 293 cells, because 293 cells used as host cells derived from the kidney.

Further, recombinant cell lines continuously expressing PSEC67 were established by selecting overexpressing clones using G-418. The cell lines were tested for the degree of oleic acid incorporation, and the oleic acid incorporation was revealed to be enhanced significantly as compared with the mock-transfected clone (Figure 3).

[Example 6] Examination of tissue distribution

The tissue distribution of PSEC67 expression in human normal tissues was investigated by PCR. Multiple Tissue cDNA Panel (CLONTECH) was used as a set of cDNAs from human normal tissues. PCR was carried out with La Taq polymerase (TaKaRa Shuzo). Primers used were as follows:

5'-oligo; 5'-AACAGGGCTGCACGCGCCTT-3' (SEQ ID NO: 11)

3'-oligo; 5'-CGGGATCCCACCTGCAACGGCCCC CACCCCACAGAGTTC-3' (SEQ ID NO: 12))

The number of cycles in the PCR experiment was 25, 30 and 35. The expression was assessed based on the result with the cycle number (30 cycles) where the band intensity was not saturated.

The test for tissue distribution by PCR revealed that PSEC67 was expressed in tissues such as the prostate gland, small intestine, placenta, testis, pancreas, peripheral blood, spleen, and liver. In addition, low-level expression of PSEC67 was also detected in the brain, heart, kidney, and large intestine (Figure 4). These findings suggest that the protein of the present invention, PSEC67, participates in the incorporation of fatty acids by the respective tissues, and eventually provides energy to the cells of the respective tissues,

as well as being involved in the uptake of fatty acids via the small intestine. In addition, the results suggest that PSEC67 also participates in the immune system.

[Example 7] Examination of the fatty-acid selectivity of PSEC67

The selectivity of PSEC67 toward long chain fatty acids was tested as follows. Namely, when BSA-bound fatty acids were prepared with the radioisotope-labeled long chain fatty acids in Example 4, BSA-bound fatty acids were also prepared with unlabeled fatty acids. Specifically, non-labeled long chain fatty acids with a 20 times higher concentration than radioisotope-labeled long chain fatty acids were prepared. Then, fatty acid-non-containing BSA was added thereto at a final molar ratio of 1:1 in the case of oleic acid, or at a final molar ratio of 1:10 in the case of arachidonic acid. The mixture was stirred gently and then twice as much volume of PBS(-) was added thereto. The mixture was incubated at 37°C for 45 minutes.

After a cell suspension was prepared and this was pre-incubated at 37°C for five minutes, 50 µl of a non-labeled fatty acid/BSA solution was added thereto. The mixture was further incubated at 37°C for 3 minutes. Then, 50 µl of radioisotope-labeled albumin-bound fatty acid was added thereto, and the mixture was allowed to react for 3 minutes. After the reaction, 5 ml of a stop solution (= Washing solution; 1% BSA (without fatty acid), 200 µM phloretin in PBS(-)) pre-cooled on ice was added to each tube to stop the reaction. Subsequent procedures were the same as in Example 4.

The results obtained are shown in Figure 5. The incorporation of both oleic acid and arachidonic acid was significantly enhanced in cells to which PSEC67 had been introduced as compared to mock-transfected cells. Non-labeled oleic acid significantly suppressed the incorporation. However, glucose, which is also transported into cells by a transporter, did not suppress fatty acid incorporation. The result described above revealed that the protein of the present invention exhibited selectivity in the transport of long chain fatty acids such as oleic acid and arachidonic acid. Further, it was assumed that there were slight or no difference in the selectivity between long chain fatty acids.

Industrial Applicability

The present invention provides novel membrane proteins associated with the metabolism of long chain fatty acids, particularly the incorporation of long chain fatty acids into cells, and the genes encoding the proteins. The proteins of the present invention, the genes encoding the proteins and compounds regulating the incorporation activity are expected to be applicable as preventive or therapeutic agents for various diseases associated with metabolic disorders of long chain fatty acids (metabolic diseases such as diabetes, arteriosclerosis and obesity, cardiomyopathy, skeletal muscle disorders, renal disorders, etc.).